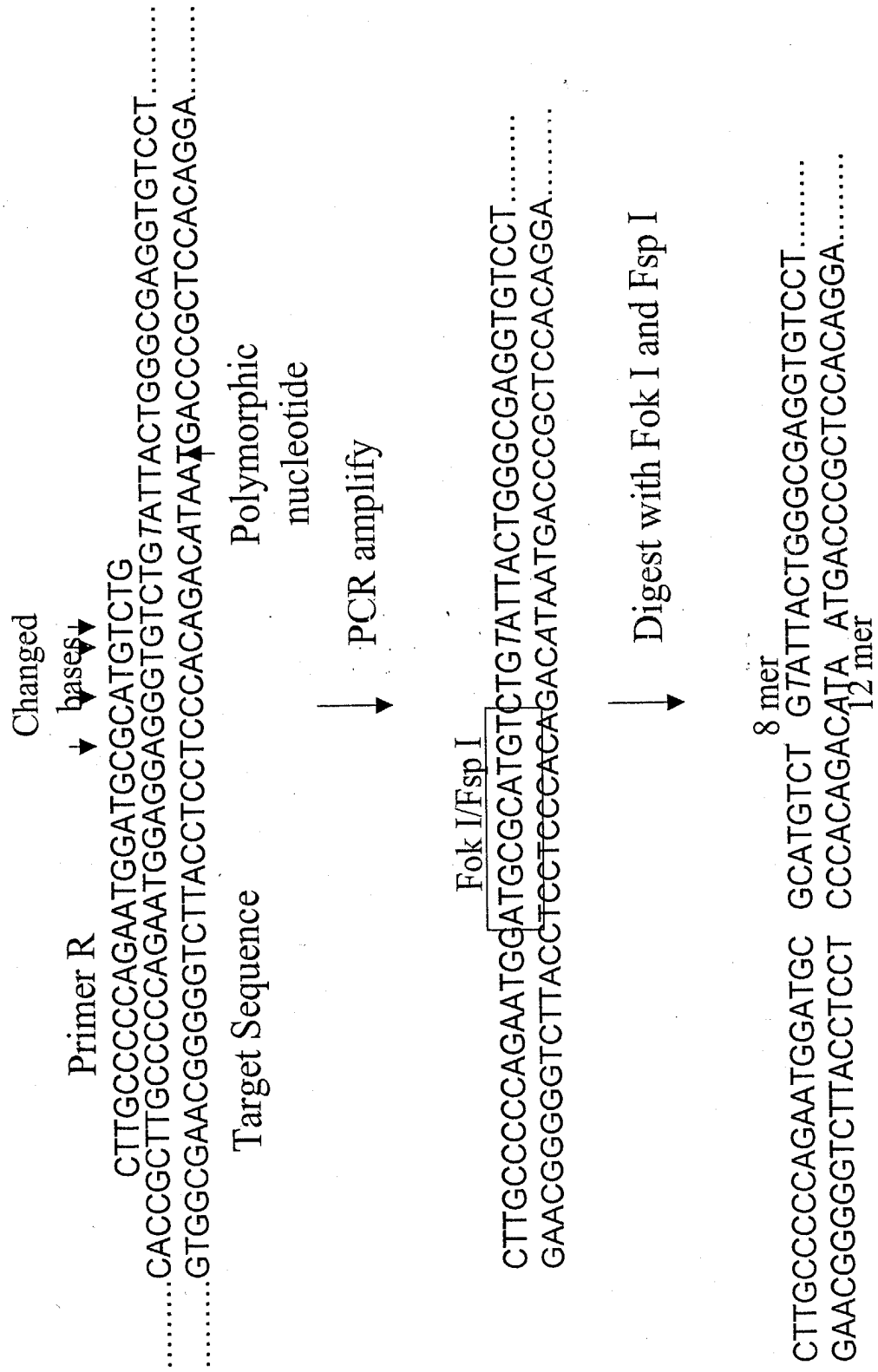


Figure 1



[illegible]

nnnnnGGATGnnnnnnnnnnnnnnnnnnnnnn
nnnnnCCGACnnnnnnnnnnnnnnnnnnnnnn

Cut with Fok I

nnnnnGGATGnnnnnnnnnn nnnnnnnnnnn
nnnnnCCGACnnnnnnnnnnnnnnnnnnnnnnnn

Fsp1

nnnnnnTGGCAnnnnn
nnnnnnACGGTnnnnn

Cut with Fsp I

nnnnnnTGC	GCAnnnnnn
nnnnnnACG	CGTnnnnnn

Figure 3



Figure 5

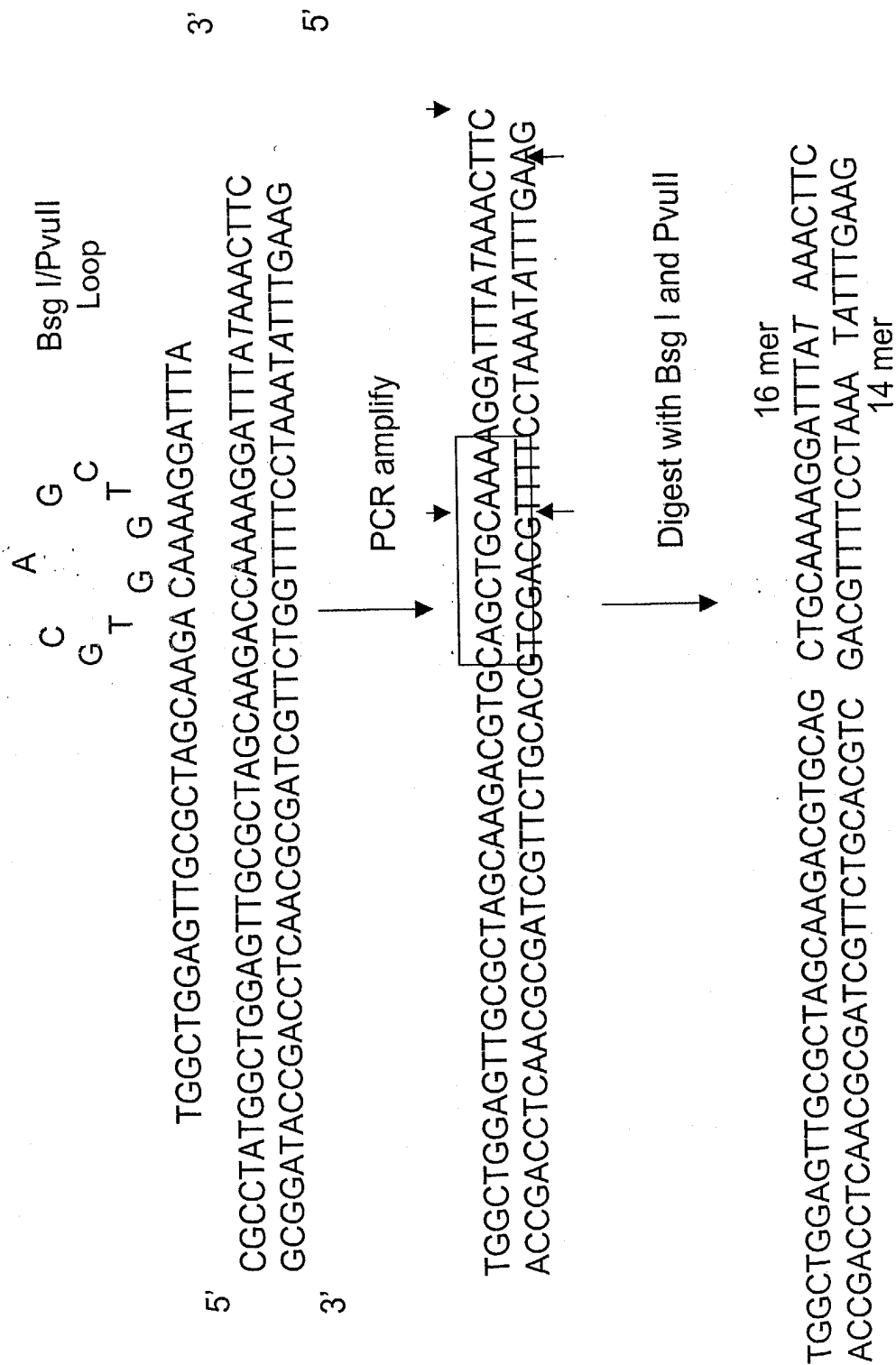


Figure 6

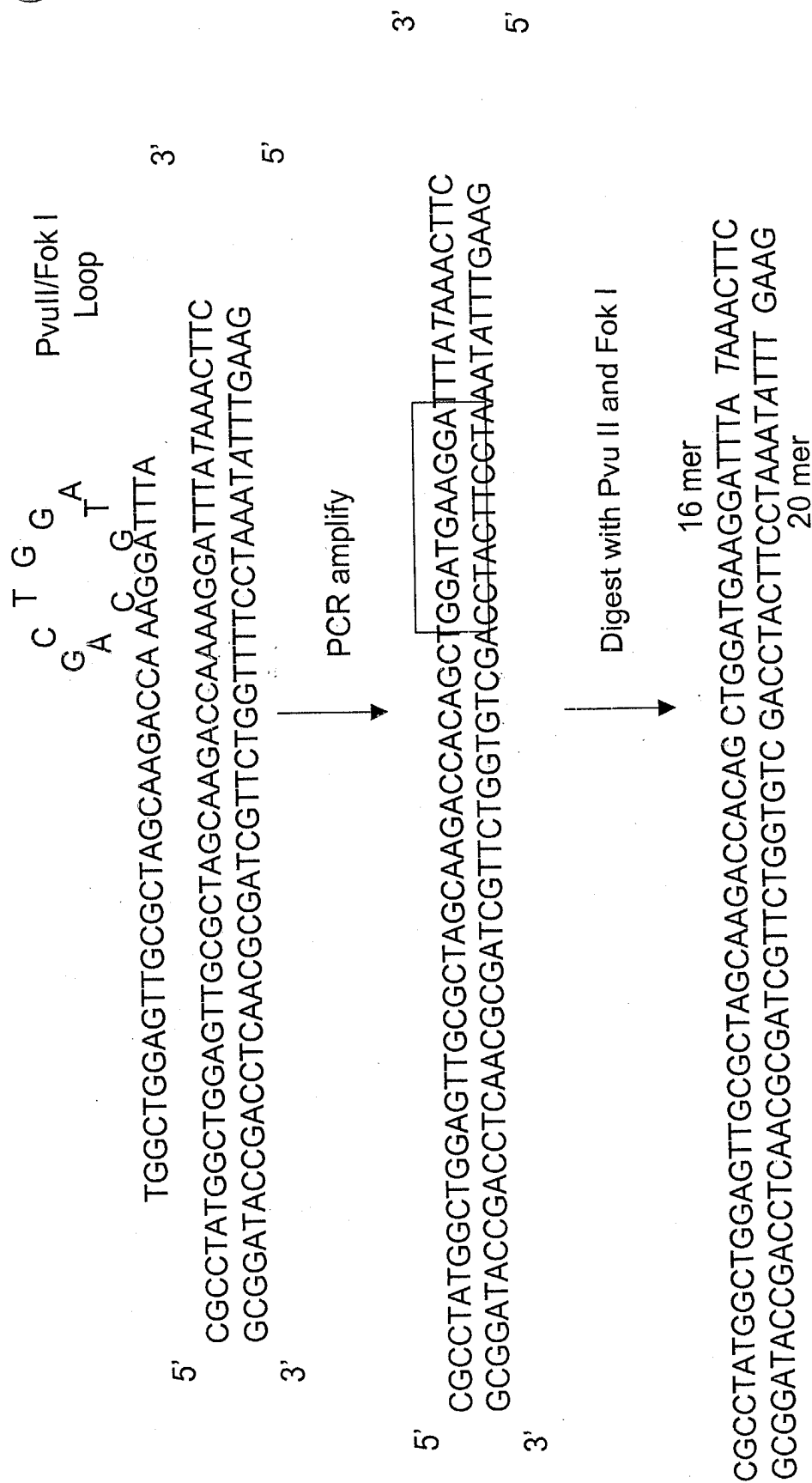


Figure 7

Fok I/Fsp I

CTTGCCCCAGAAATGGAGGAGGATGCGCAGGTGTCTGTATTACTGGGCGAGGT.....
GAACGGGGTCTTACCTCCTCCTACCGGTCACAGACATAATGACCCGCTCCA.....

↓
Remove nucleotides and
digest with Fok I

CTTGCCCCAGAAATGGAGGAGGATGCGCAGGTGT
GAACGGGGTCTTACCTCCTCCTACCGGTCACAGACA

↓
Fill in with mass
Modified nucleotide

CTTGCCCCAGAAATGGAGGAGGATGCGCAGGTGTCTGT^{mod}
GAACGGGGTCTTACCTCCTCCTACCGGTCACAGACA

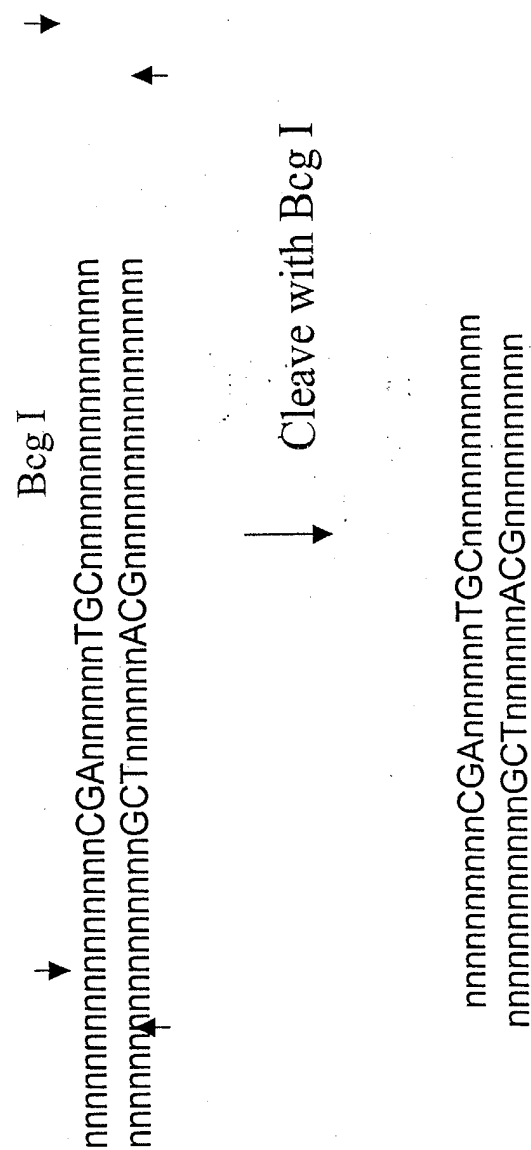
[illegible]

Figure 9

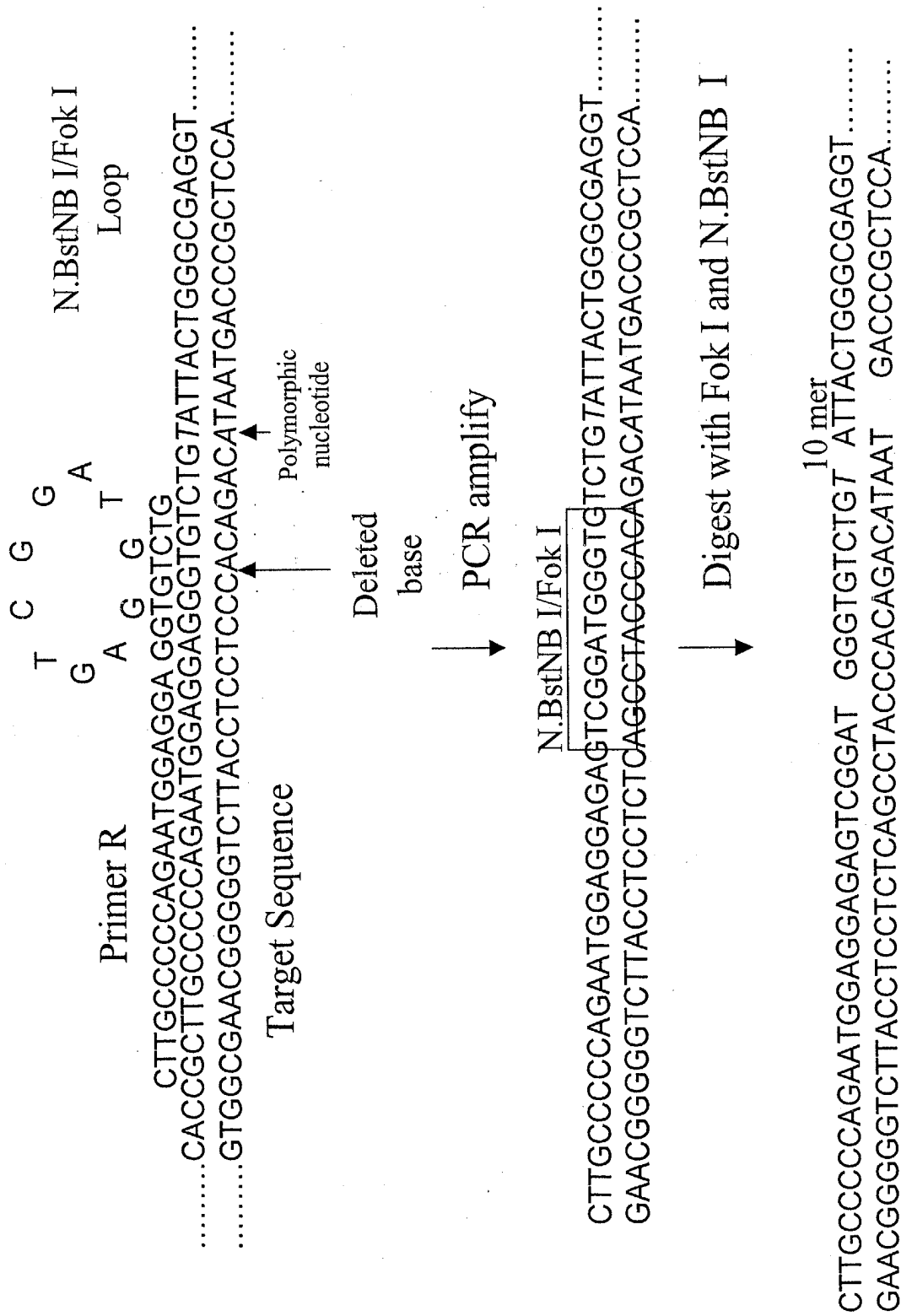


Figure 10

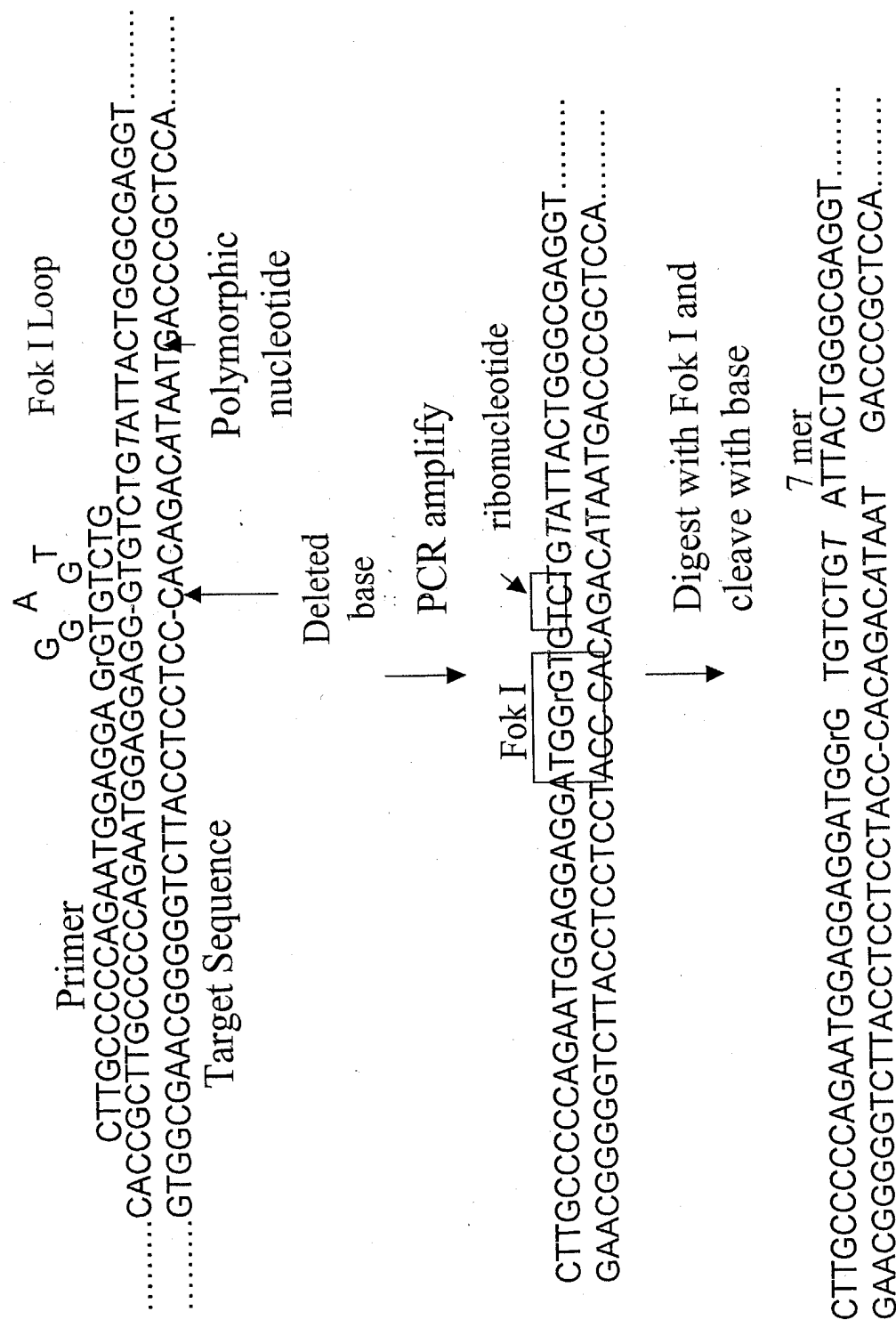


Figure 11. Methods for haplotyping based on physical allele separation

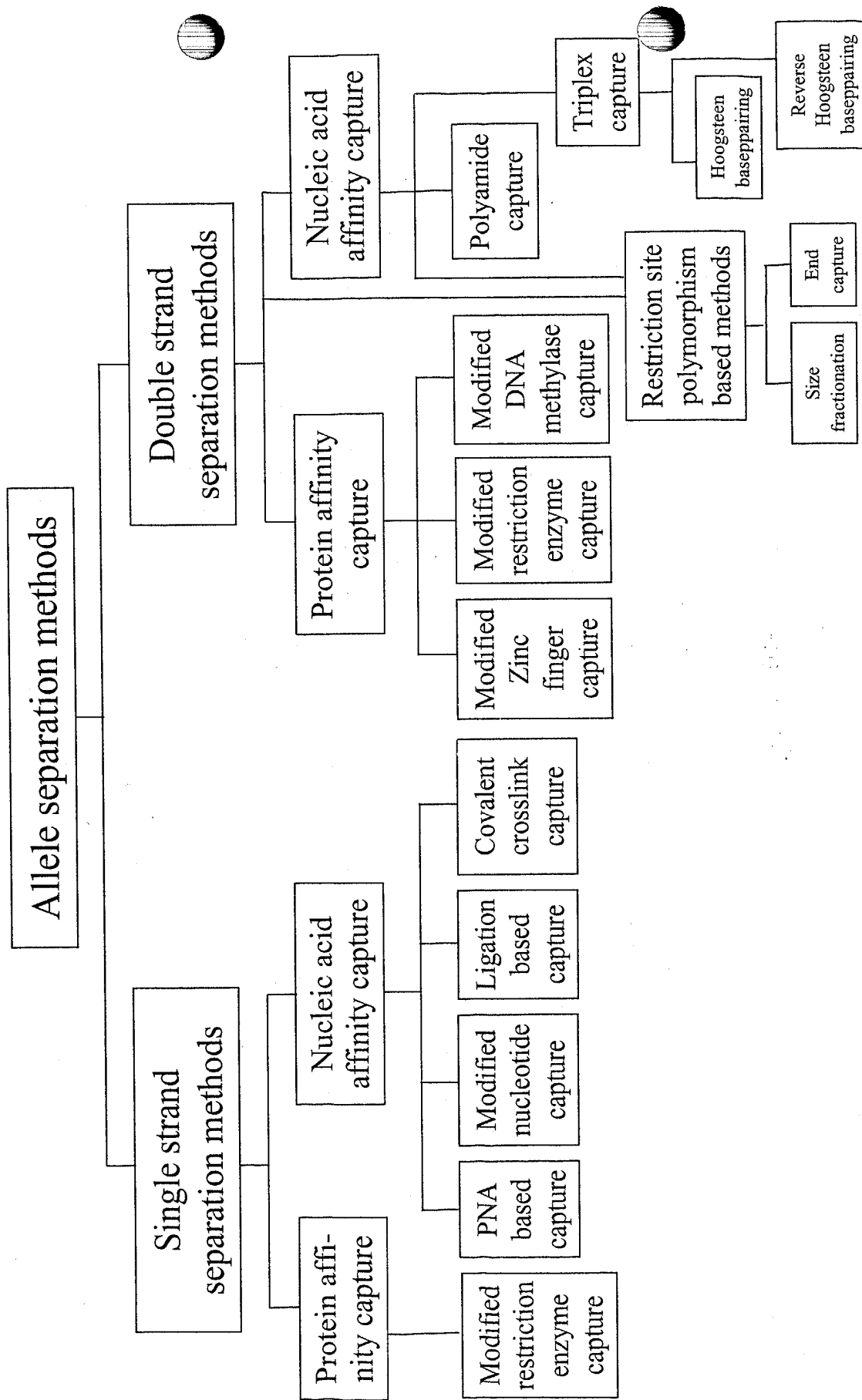


Figure 12. Methods for haplotyping based on allele specific amplification

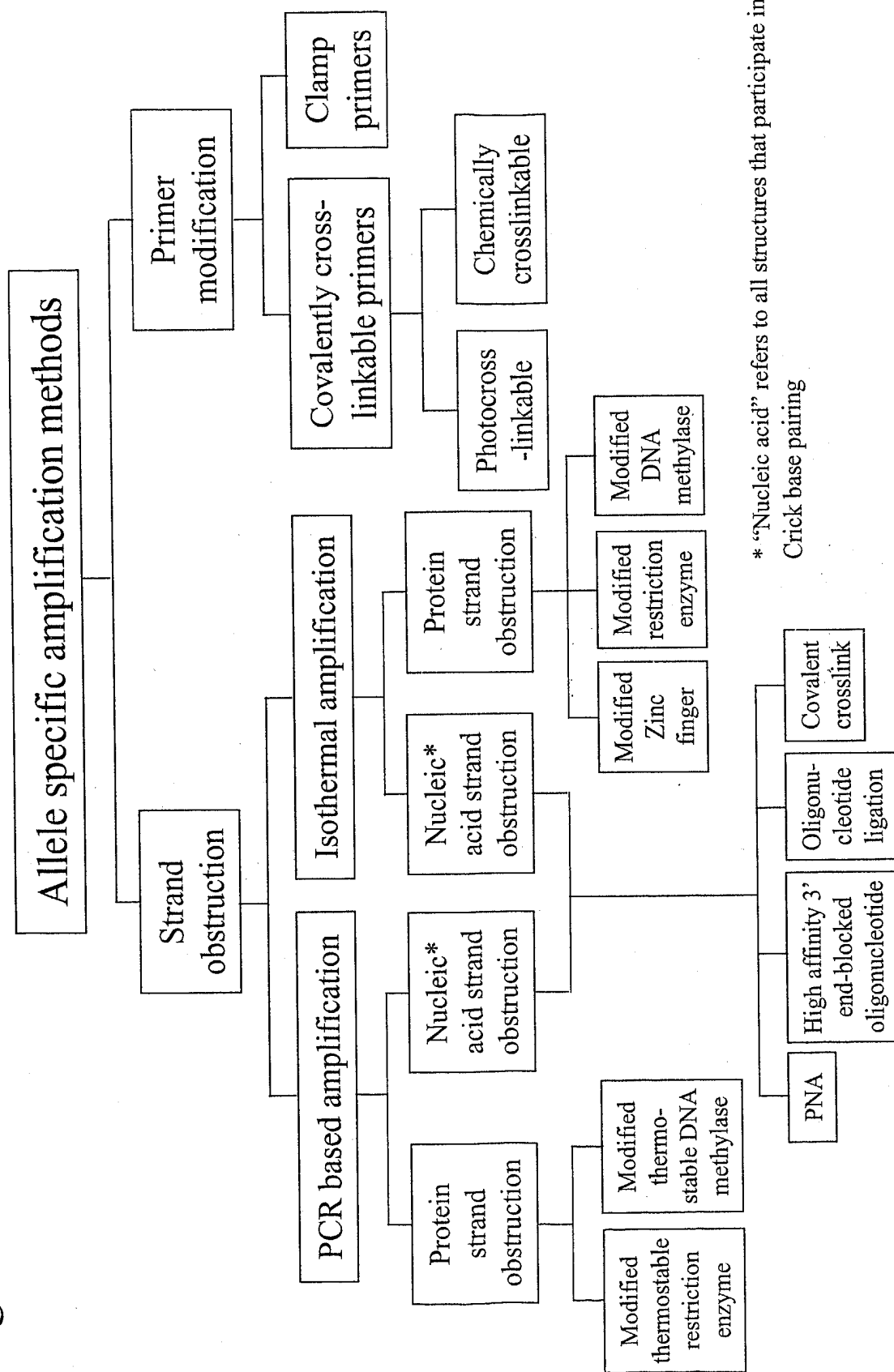


Figure 13. Methods for haplotyping based on allele specific restriction

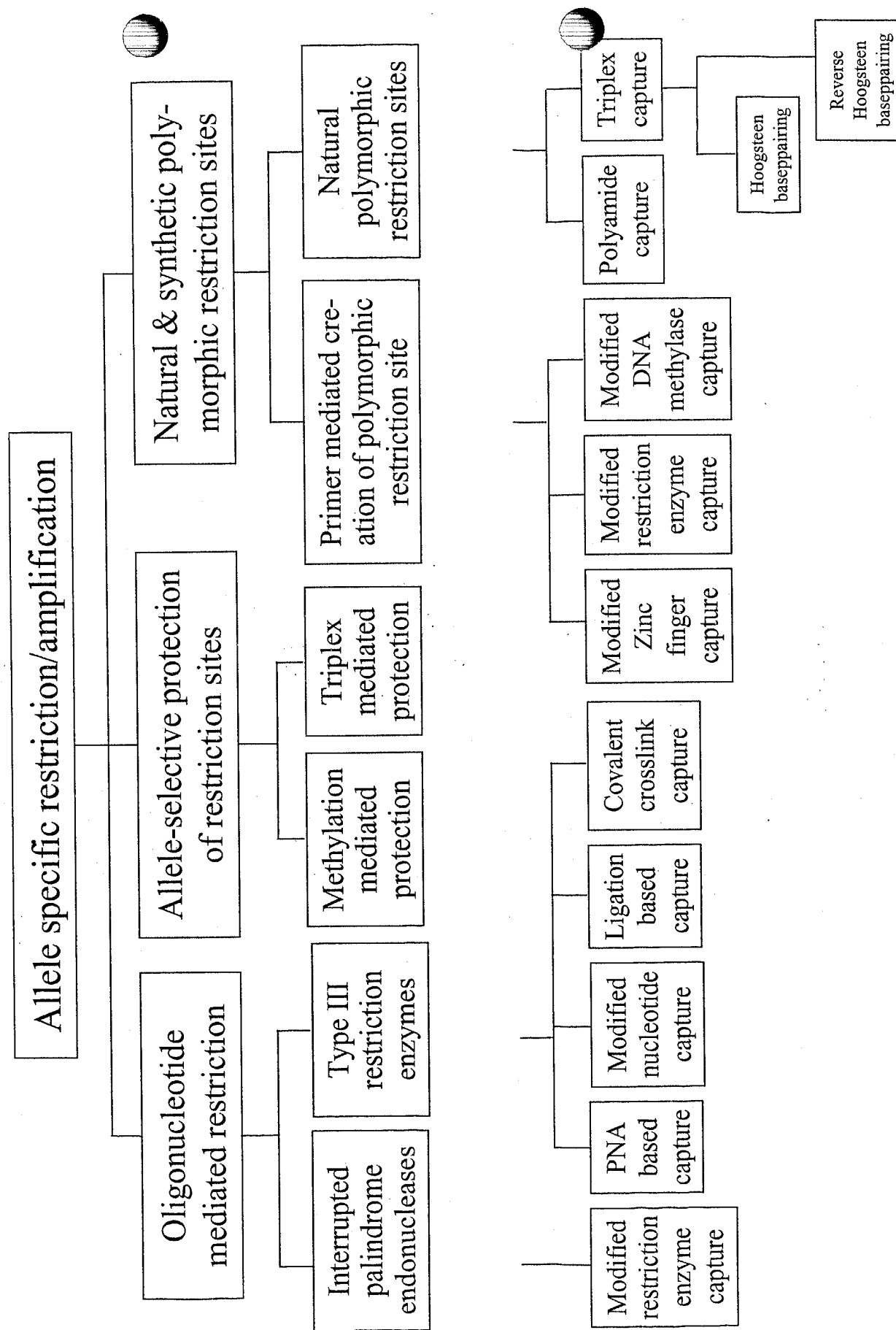


Figure 14: Hairpin PCR Primers

ATCTGGANNNNNNNNNNNNTCC

AGGTCTA

ALLELE 1
T PRIMER
↓ PCR Amplify

ATCTGGANNNNNNNNNNNNTCCAGAT

TAGACCTNNNNNNNNNNNAGGTCTA

ATCTGGANNNNNNNNNNNNTCC

AGGCCTA

ALLELE 2
T PRIMER
↓ PCR Amplify

ATCTGGANNNNNNNNNNNNTCCGGAT

TAGACCTNNNNNNNNNNNAGGCCTA

Figure 15: Hairpin PCR Primers

ATCCGGANNNNNNNNNNTCC
AGGTCTA

ALLELE 1
C PRIMER
↓ PCR Amplify

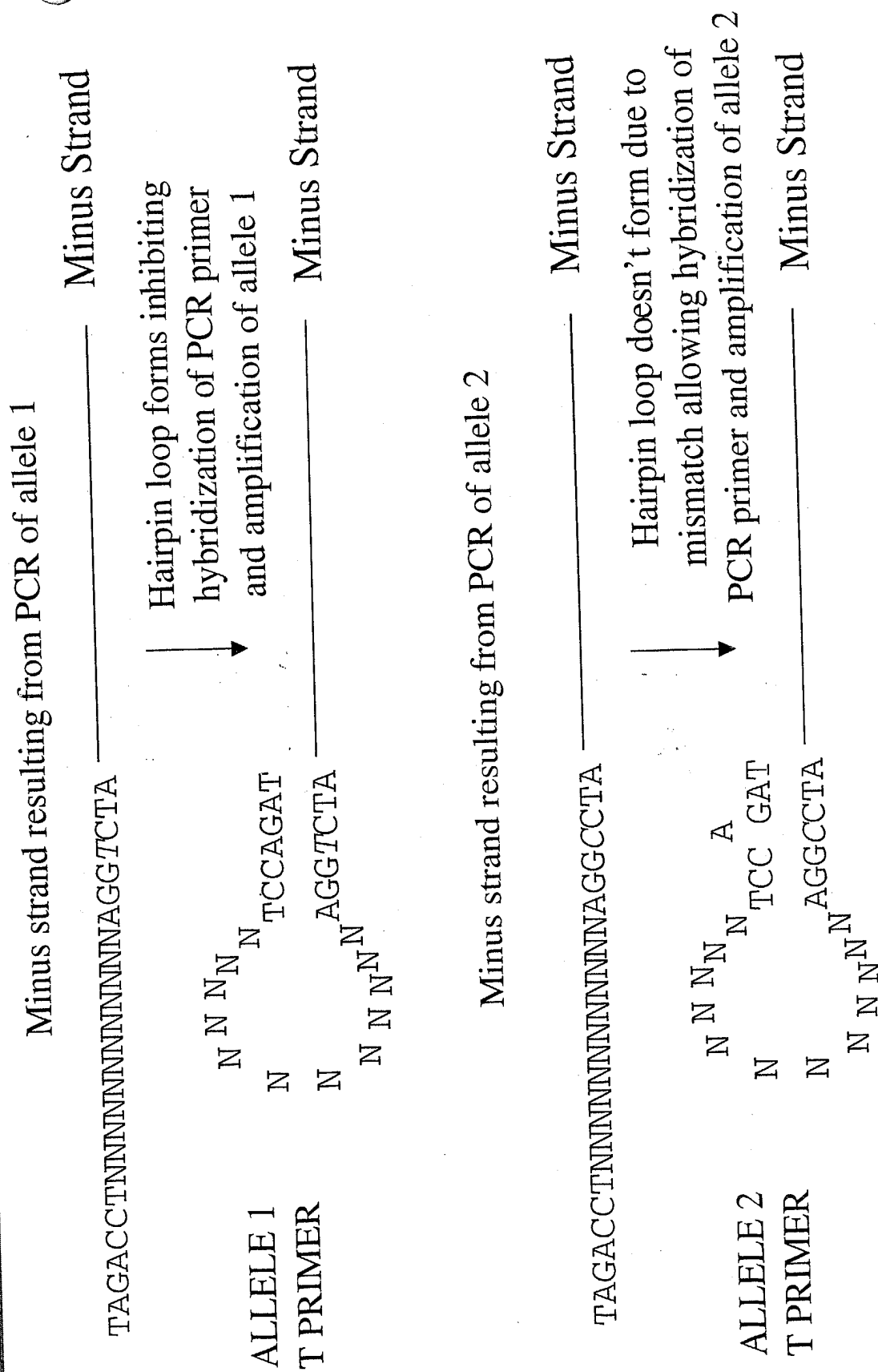
ATCCGGANNNNNNNNNNTCCAGAT
TAGGCCTNNNNNNNNNNNAGGTCTA

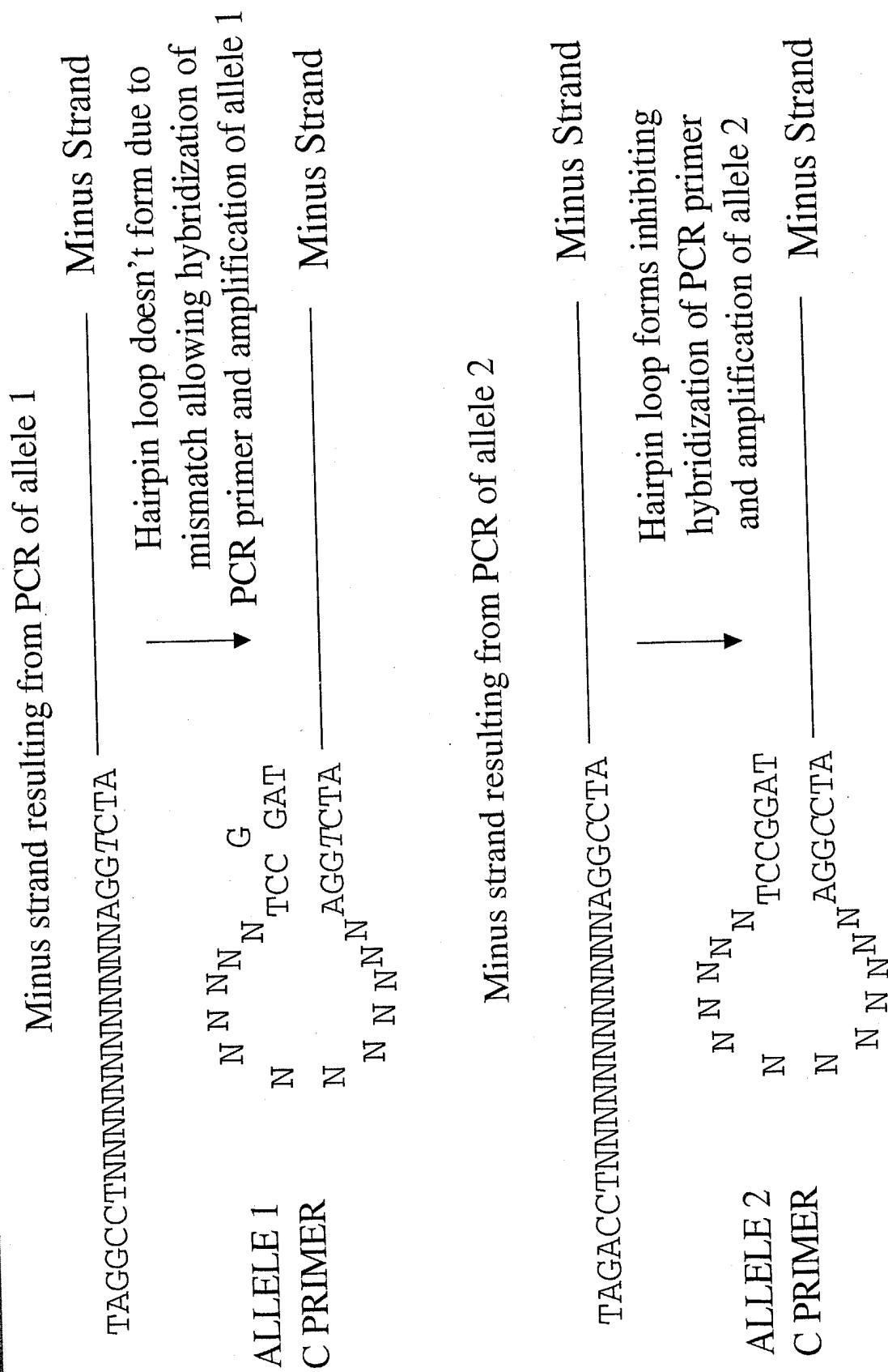
ATCCGGANNNNNNNNNNTCC
AGGCCTA

ALLELE 2
C PRIMER
↓ PCR Amplify

ATCCGGANNNNNNNNNNTCCGGAT
TAGGCCTNNNNNNNNNNNAGGCCTA

Figure 16: Hairpin PCR Primers





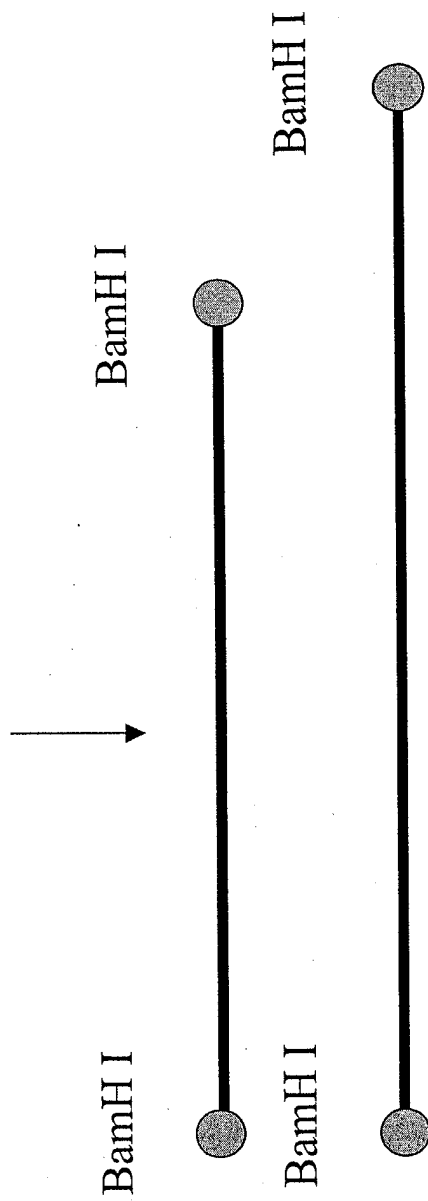
[illegible]

Diagram illustrating the BamHI recognition and cleavage sites on DNA. The top strand shows a BamHI site (GGATCC) with a cleavage site indicated by a vertical line between the G and G. The bottom strand shows a BamHI site (GGATCC) with a cleavage site indicated by a vertical line between the G and G. An arrow points to the cleavage site on the top strand, labeled "Polymorphic Site".

→ re →

Figure 19

Protect ends from exonuclease digestion



Restrict with second enzyme

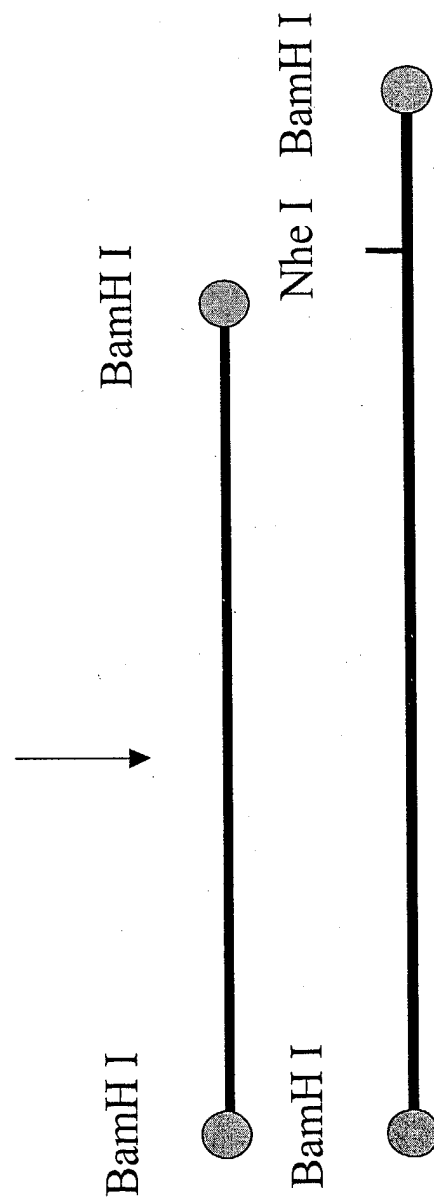


Figure 20

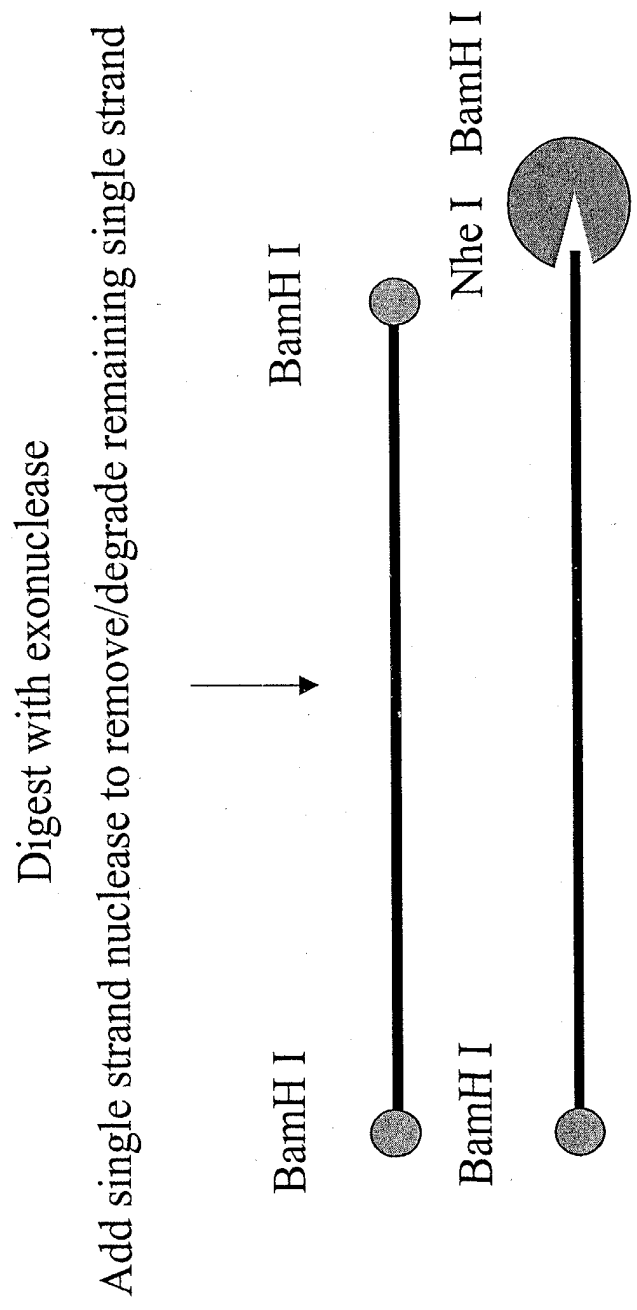


Figure 21. Dihydropyrimidine dehydrogenase (DPD) polymorphisms used in haplotyping assay.

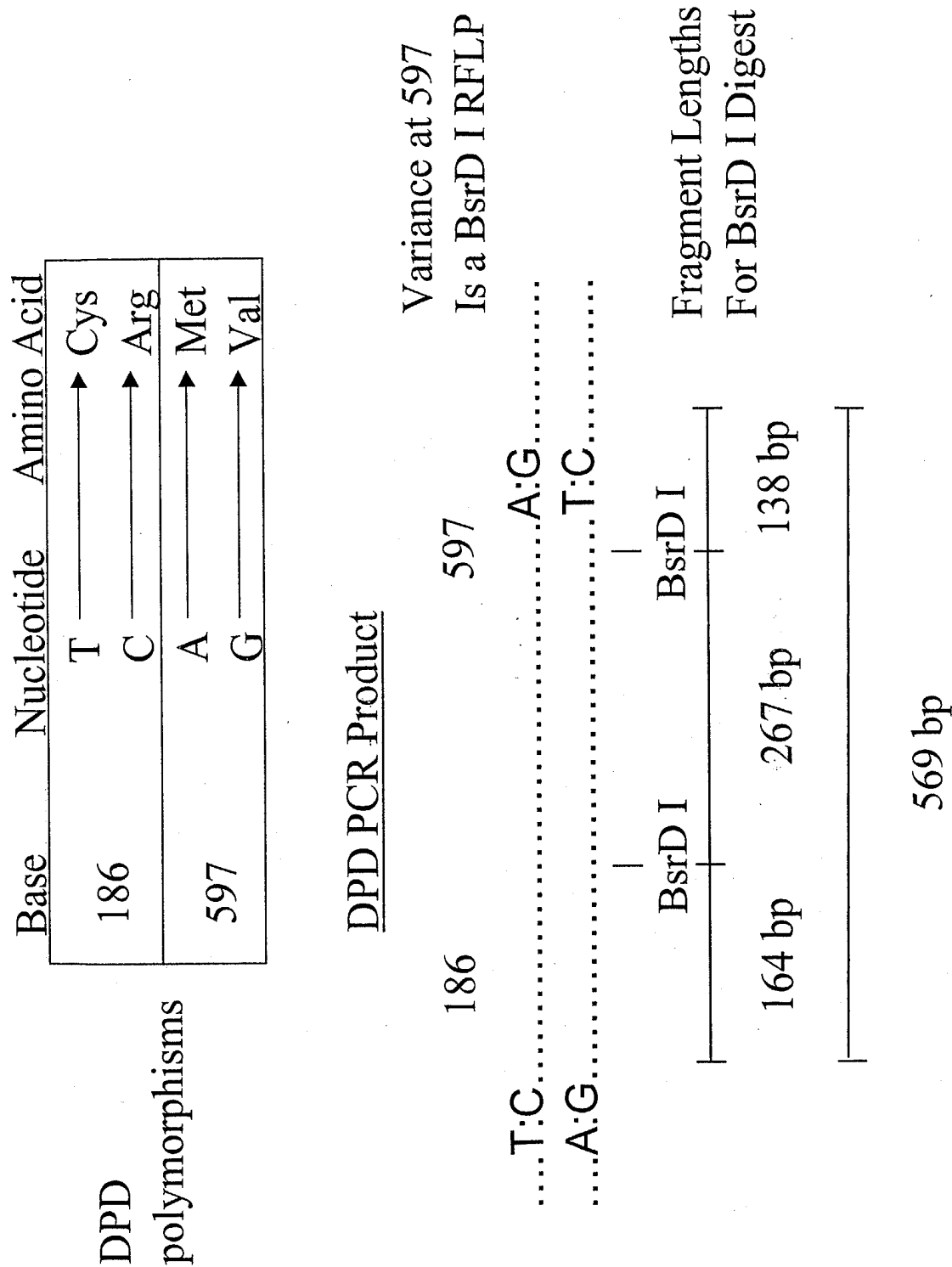


Figure 22. Allele Specific Primers for DPD

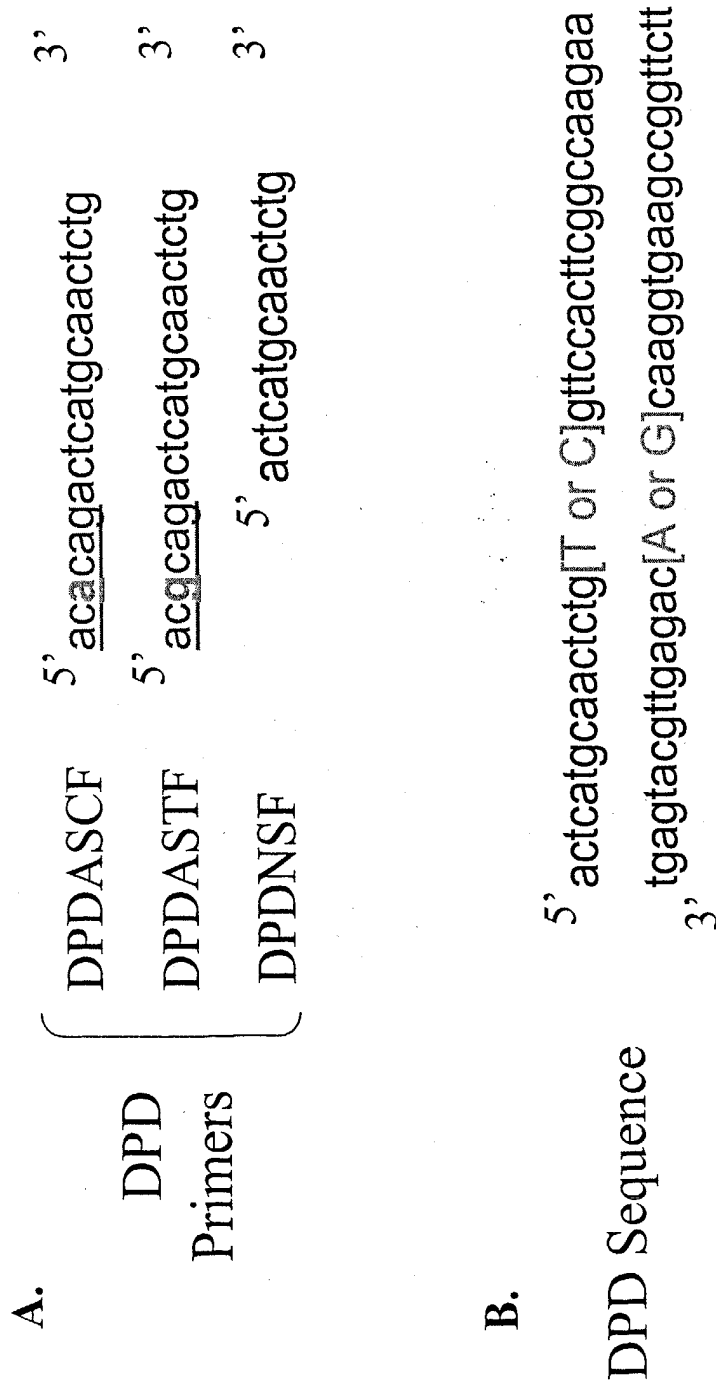


Figure 23. PCR Amplification Using DPDNSF Primer

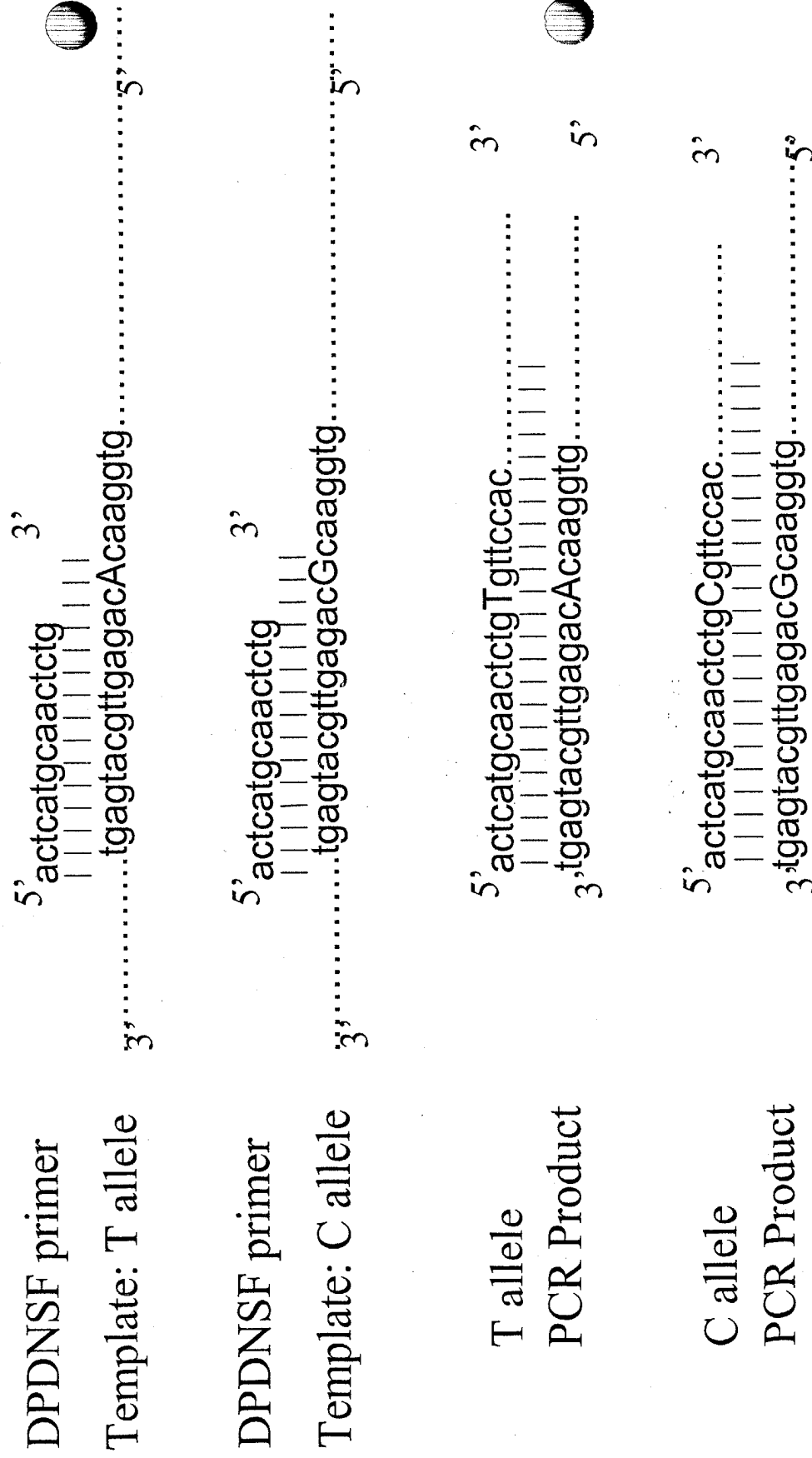


Figure 24. PCR Amplification Using DPDASTF Primer.

DPDASTF primer

5' acgcagactcatgcaactctg 3'

Template T allele

3'tgatcgttgagacAcaaggtag 5'

DPDASTF primer

5' acgcagactcatgcaactctg 3'

Template C allele

3'tgatcgttgagacGcaaggtag 5'

T allele

5' acgcagactcatgcaactctgTgtccac 3'

PCR Product

3' tgcatctgagtagcgttgagacAcaaggtag 5'

C allele

5' acgcagactcatgcaactctgGgtccac 3'

PCR Product

3' tgcatctgagtagcgttgagacGcaaggtag 5'

Figure 25. PCR Amplification Using DPDASCF Primer

DPDASCF primer

5' acacagactcatgcaactctg ||||| 3'

Template T allele

3'tgagtacgttgagacAcaagggtg..... 5'

DPDASCF primer

5' acacagactcatgcaactctg ||||| 3'

Template C allele

3'tgagtacgttgagacGcaagggtg..... 5'

T allele

5' acacagactcatgcaactctg Tgttccac..... 3'

PCR Product

3' tgtgtctgagtacgttgagacAcaagggtg..... 5'

C allele

5' acacagactcatgcaactctg Cgttccac..... 3'

PCR Product

3' tgtgtctgagtacgttgagacGcaagggtg..... 5'

Figure 26. Hairpin Structures for PCR Products Generated Using DPDNSF Primer

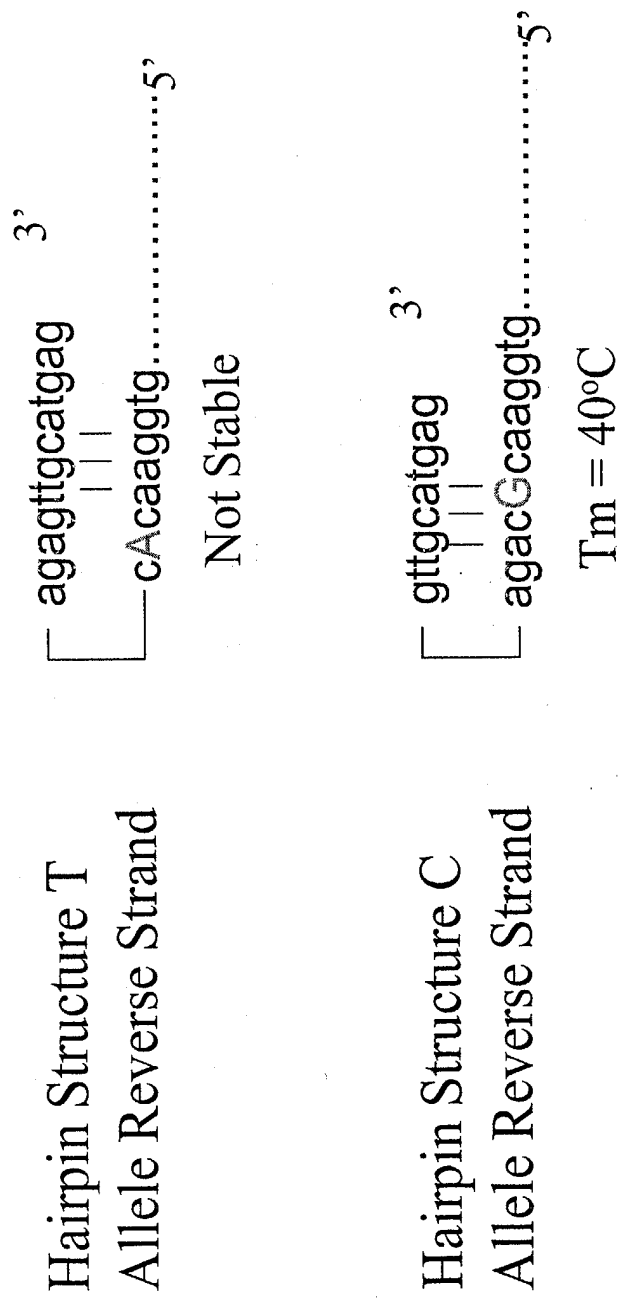


Figure 27. Hairpin Structures for PCR Products Generated Using DPDASCF Primer

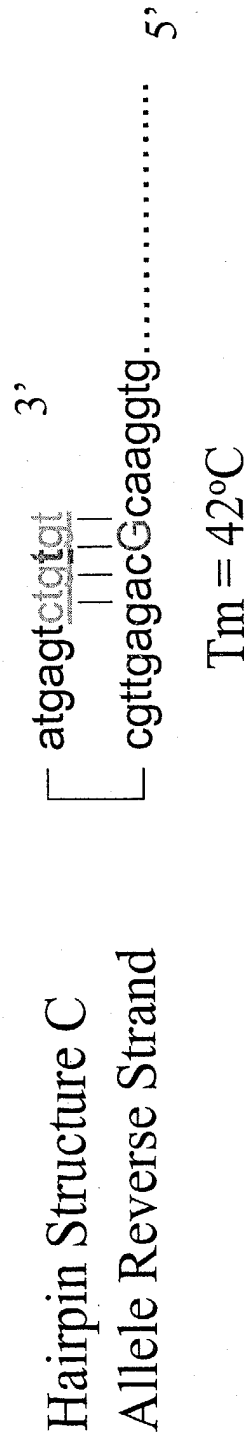
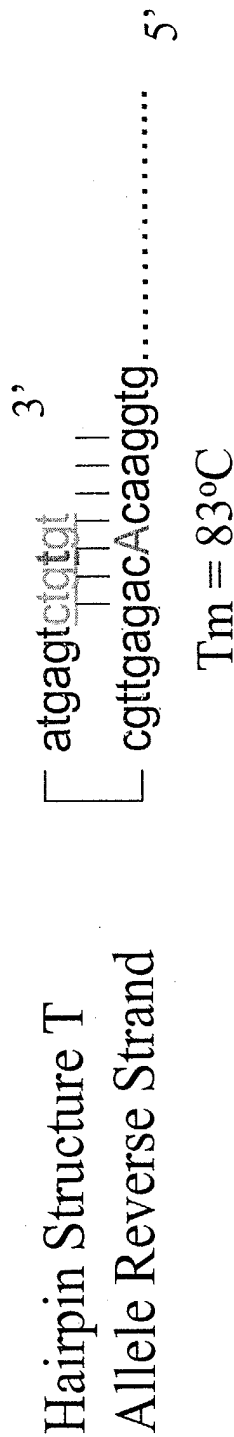


Figure 28. Hairpin Structures for PCR Products Generated Using DPDASTF Primer

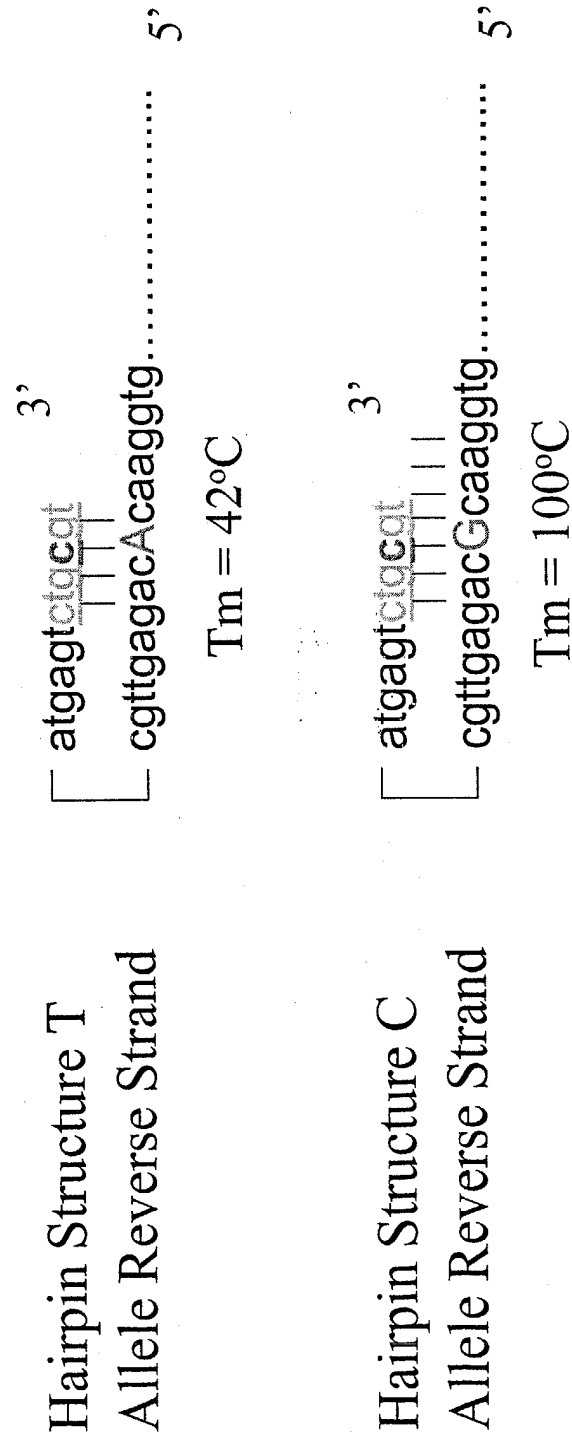


Figure 29. Non-Allele Specific Amplification Using DPDNSF Primer.

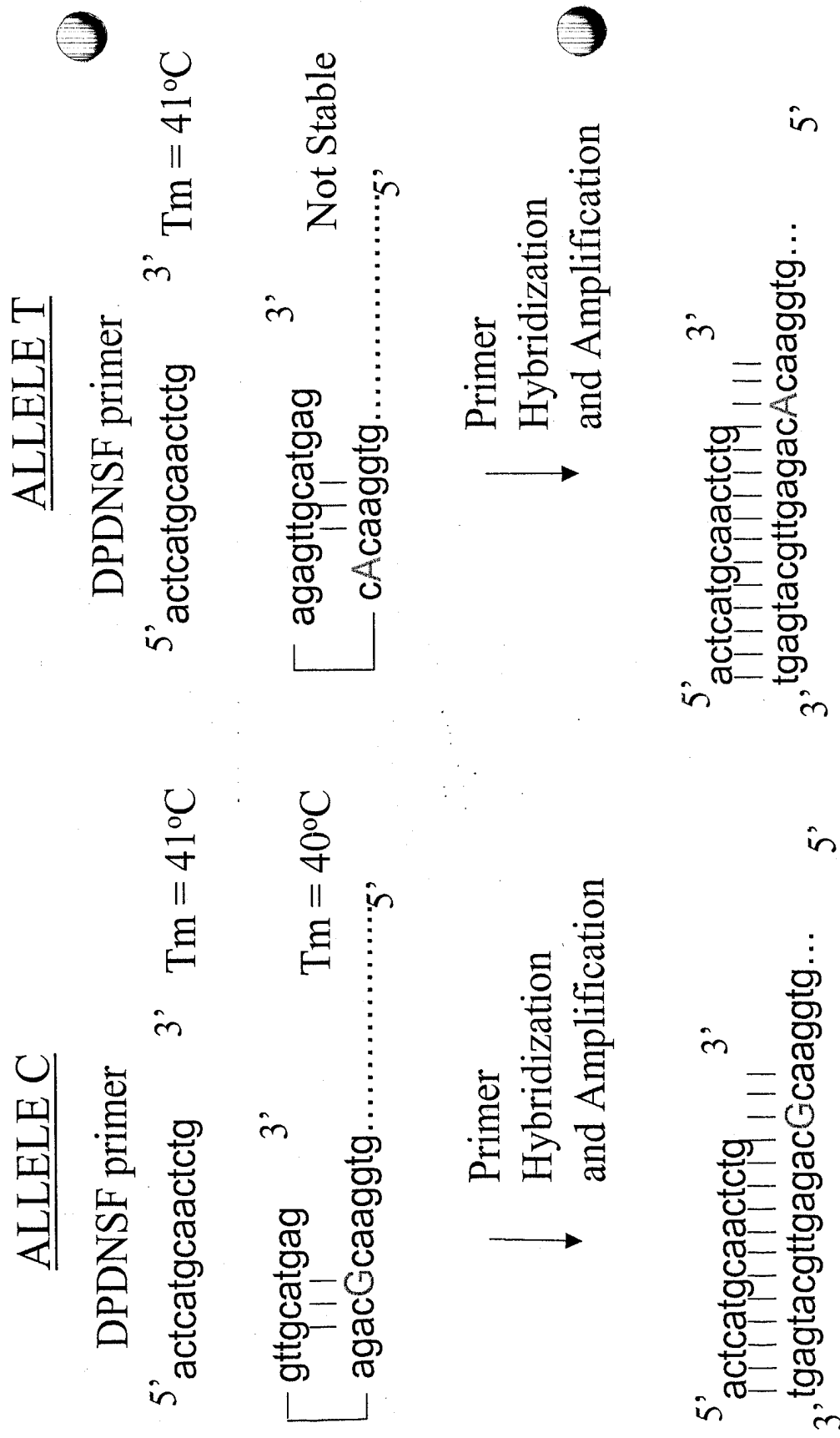


Figure 30. Allele Specific Amplification Using DPDASCF Primer

ALLELE C

DPDASCF primer $T_m = 60^\circ\text{C}$

5' acacagactcatgcaactctg 3'

$\left[\begin{array}{c} \text{atgagtcctat} \\ |||| \\ \text{cgtagagacGcaagg} \end{array} \right] \dots 5'$
 $T_m = 42^\circ\text{C}$

Primer
Hybridization
and Amplification

5' acacagactcatgcaactctg 3'
 3' tctctctgagtagcgtagacGcaagg... 5'

ALLELE T

DPDASCF primer $T_m = 60^\circ\text{C}$

5' acacagactcatgcaactctg 3'

$\left[\begin{array}{c} \text{atgagtcctat} \\ |||| \\ \text{cgtagagacAcaagg} \end{array} \right] \dots 5'$
 $T_m = 83^\circ\text{C}$

Hairpin inhibits
Primer Hybridization
and Amplification

5' acacagactcatgcaactctg 3'
 3' atgagtcctat 3'
 5' cgtagagacAcaagg... 5'

Figure 31. Allele Specific Amplification Using DPDASTF Primer

ALLELE C

DPDASTF primer $T_m = 65^\circ\text{C}$

5' acgcagactcatgcaactctg

[atgagtctacat
|||||
cgttgagacGcaaggtag..... 5'

$T_m = 100^\circ\text{C}$

Hairpin inhibits
primer hybridization
and Amplification

5' acgcagactcatgcaactctg 3'

[atgagtctacat
|||||
cgttgagacGcaaggtag..... 5'

ALLELE T

DPDASTF primer $T_m = 65^\circ\text{C}$

acgcagactcatgcaactctg

[atgagtctacat
|||||
cgttgagacAcaaggtag..... 5'

$T_m = 42^\circ\text{C}$

Primer hybridizes
↓
and amplification ensues

5' acgcagactcatgcaactctg 3'
|||||
cgttgagacAcaaggtag..... 5'

Figure 32. Allele Specific Amplification of a Heterozygous Sample with Haplotype T¹⁸⁶A⁵⁹⁷ and C¹⁸⁶G⁵⁹⁷

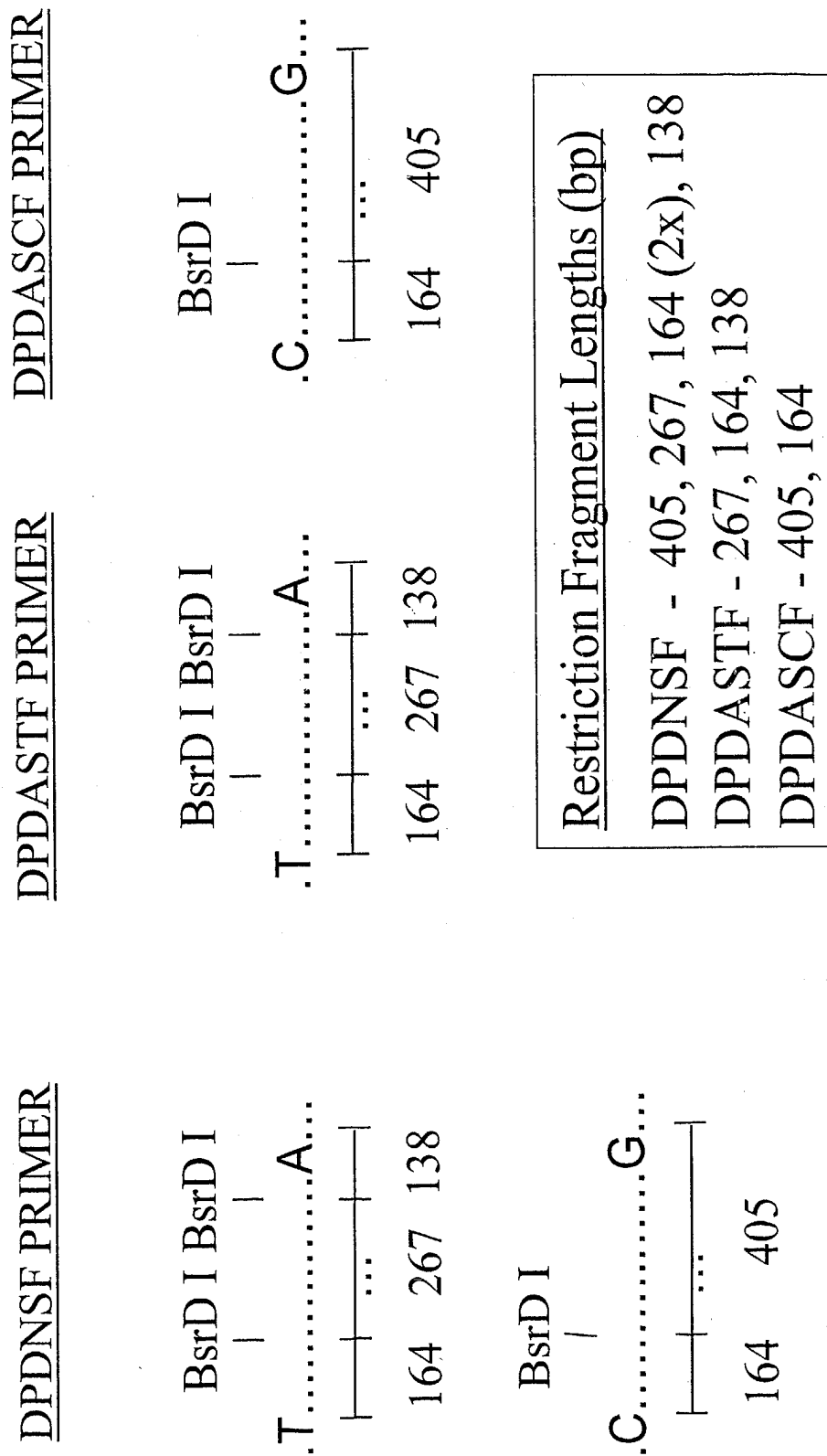


Figure 33. BsrD I Digest of Allele Specific PCR Products.

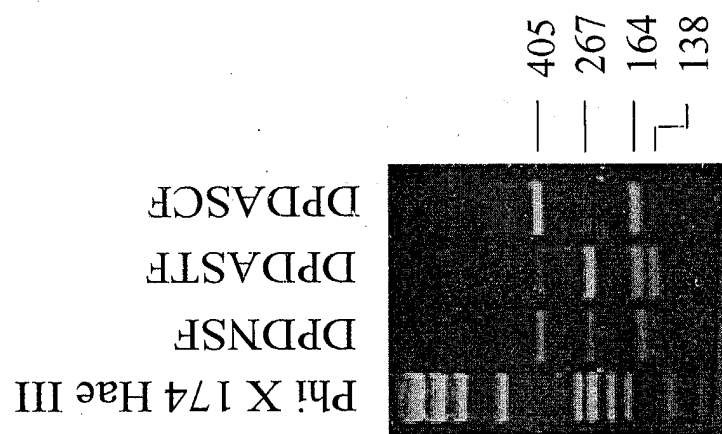


Figure 34

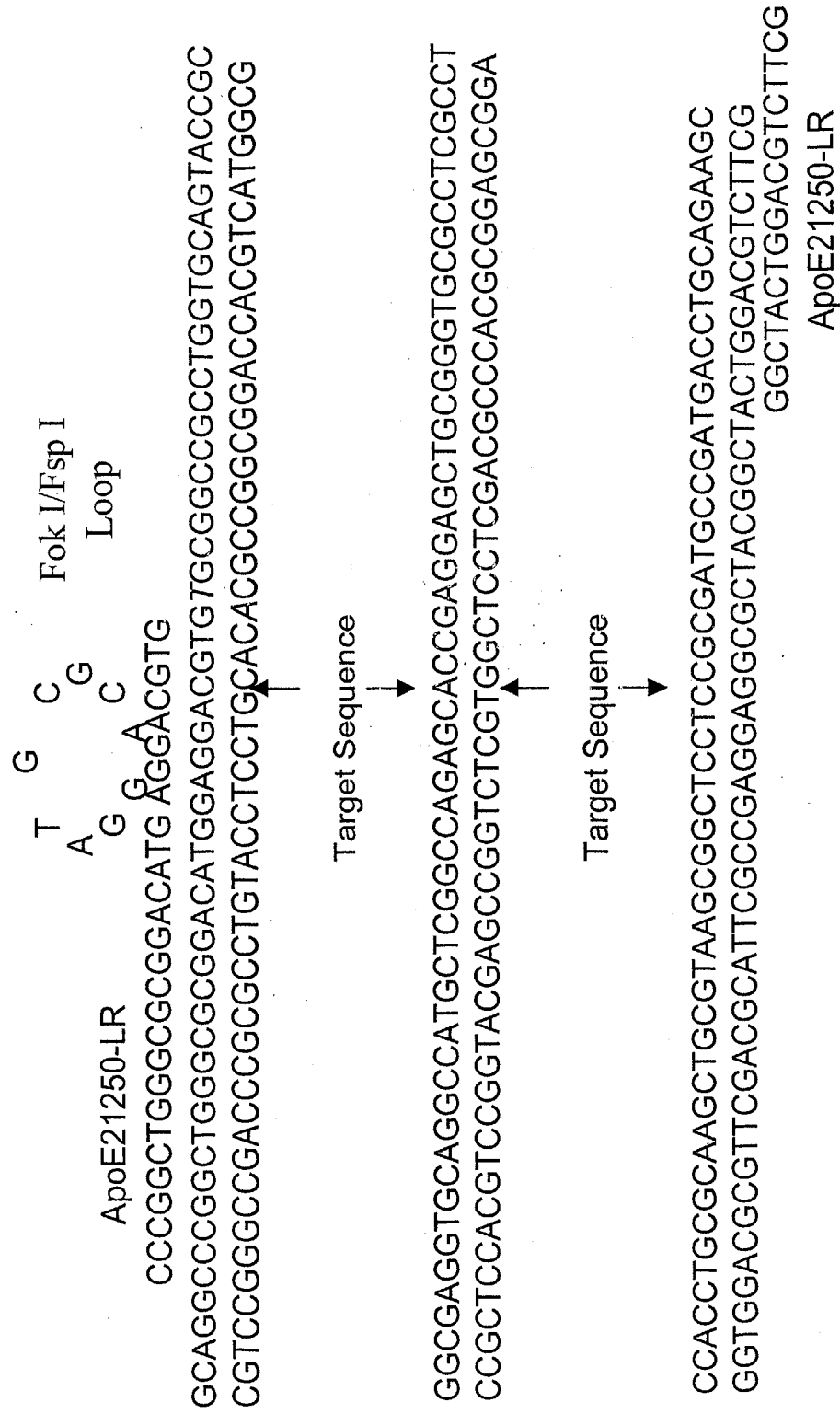


Figure 35

T Allele Amplicon

↓ ↓
CCCGGCTGGCGCGGACATGGGATGCGCAAGGACGTGTGCGGGCCGCCTGGTGCAGTAC
GGCGGACCCGCGCCTGTACCTACGCGTTCCTGCGACACGCCCGCGGACCCACGTCATG

CGCGGCGAGGTGCAGGCCATGCTCGGCCAGAGACCGAGAGCTGCGGGTGCGCCTCG
GGCCCGCTCCACGTCCGGTACGAGCCGGTCTCGTGGCTCCTCGACGCCACCGGGAGC

CCTCCACCTGCGCAAGCTGCGTAAGCGGCTCCTCCGGATGCCGATGACCTGCAGAAGC
GGAGGTGGACGCGTTCCGACGCATTCCGCCGAGGAGCGCTACGGCTACTGGACGTCTTCG

C Allele Amplicon

↓ ↓
CCCGGCTGGCGCGGACATGGGATGCGCAAGGACGTGCGGGCCGCCTGGTGCAGTAC
GGCCGACCCGCGCCTGTACCTACGCGTTCCTGCGACGCGCCCGCGGACCCACGTCATG

CGCGGCGAGGTGCAGGCCATGCTCGGCCAGAGACCGAGAGCTGCGGGTGCGCCTCG
GCGCCGCTCCACGTCCGGTACGAGCCGGTCTCGTGGCTCCTCGACGCCACCGGGAGC

CCTCCACCTGCGCAAGCTGCGTAAGCGGCTCCTCCGGATGCCGATGACCTGCAGAAGC
GGAGGTGGACGCGTTCCGACGCATTCCGCCGAGGAGCGCTACGGCTACTGGACGTCTTCG